

Preliminary Amendment to Claims

Dear Examiner,

We hereby request the following amendments be made to replace all previous claims to of Method for Improving the Agronomic and Nutritional Value of Plants International Application Number PCT/EP00/01850.

Signed *Jay Baker* Date *Sept 4, 2001*
#41,595

1. Isolated DNA molecules comprising nucleotide sequences comprising one or more expression cassettes capable of directing expression of two or three enzymes characteristic for the carotenoid biosynthesis pathway in plant cells selected from the group consisting of:
phytoene synthase derived from a plant and phytoene desaturase derived from fungi;
phytoene synthase derived from a plant and phytoene desaturase derived from bacteria;
phytoene synthase derived from fungi and phytoene desaturase derived from fungi;
phytoene synthase derived from fungi and phytoene desaturase derived from bacteria;
phytoene synthase derived from a plant, phytoene desaturase derived from a plant and ζ -carotene desaturase derived from a plant; and,
phytoene synthase derived from fungi, phytoene desaturase derived from a plant and ζ -carotene desaturase derived from a plant.

2. The DNA molecules according to claim 1 wherein the expression cassettes comprise suitable constitutive, inducible or tissue-specific promoters operably linked to encoding sequences for the enzymes allowing expression in plant cells, seeds, tissues or whole plants.

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3. The DNA molecules according to claim 1 further comprising at least one selectable marker gene operably linked to a constitutive, inducible or tissue specific promoter sequence.
4. The DNA molecules according to claim 3 wherein the selectable marker is hygromycin phosphotransferase under control of a constitutive promoter.
5. The DNA molecules according to claim 1 wherein the plant derived phytoene synthase is expressed under the control of a tissue-specific promoter.
6. The DNA molecules according to claim 1 wherein the nucleotide sequences further comprise an encoding sequence for phytoene desaturase fused to a suitable plastid transit peptide encoding sequence.
7. The DNA molecules according to claim 6 wherein both the phytoene desaturase encoding sequence and plastid transit peptide encoding sequence are expressed under control of a tissue specific or constitutive promoter.
8. The DNA molecules according to claim 6 wherein the plastid transit peptide encoding sequence is derived from the pea Rubisco small subunit (tp).
9. The DNA molecules according to claim 1 further comprising encoding sequences consisting of a plasmid or a vector system suitable for expression in plant cells.
10. The DNA molecules according to claim 9 wherein the plasmid or vector system encoding sequence is derived from *Agrobacterium tumefaciens*.
11. The DNA molecules according to claim 1 wherein the nucleotide sequence comprises phytoene synthase derived from a plant and phytoene desaturase derived from bacteria and the plant cells are normally carotenoid free.

12. The DNA molecules according to claim 11 wherein the plant cells that are normally carotenoid free consist of rice cells.
13. A transgenic plant cell, seed, tissue or whole plant that contains one or more DNA molecules according to claim 1.
14. A transgenic plant cell, seed, tissue or whole plant according to claim 13 selected from the group consisting of eukaryotic alga, embryophytes comprising *Bryophyta*, *Pteridophyta*, *Gymnospermae*, *Magnoliopsida*, *Rosopsida*, and *Liliopsida* ("monocots").
15. A transgenic plant cell, seed, tissue or whole plant according to claim 14 selected from the group consisting of rice, wheat, barley, oats, amaranth, flax, triticale, rye, corn, *Brassica* seeds, cotton seeds, soybean, safflower, sunflower, coconut, palm, pumpkin, squash, sesame, poppy, grape, mung beans, peanut, peas, beans, radish, alfalfa, cocoa, coffee, hemp, walnuts, almonds, pecans, chick-peas potatoes, carrots, sweet potatoes, tomato, pepper, cassava, willows, oaks, elms, maples, apples, bananas, lilies, orchids, sedges, roses, buttercups, petunias, phlox, violets, and sunflowers.
16. A method of transforming plant cells, seeds or tissues using the DNA molecules according to claim 1.
17. A method of transforming plant cells, seeds or tissues according to claim 16 wherein the expression cassettes are present on one or more DNA molecules.
18. A method according to claim 16 wherein the plant cells, seeds or tissues selected for transformation are normally carotenoid free.

19. A method according to claim 18 wherein the plant cells, seeds or tissues selected for transformation have a carotenoid content 0.001% w/w or lower.
20. A transformed whole plant or part thereof regenerated from transformants yielded according to claim 16.
21. A transformed whole plant or part thereof according to claim 20 selected from the group consisting of eukaryotic alga, embryophytes comprising *Bryophyta*, *Pteridophyta*, *Gymnospermae*, *Magnoliopsida*, *Rosopsida*, and *Liliopsida* ("monocots").
22. A transformed whole plant or part thereof according to claim 21 selected from the group consisting of rice, wheat, barley, oats, amaranth, flax, triticale, rye, corn, *Brassica* seeds, cotton seeds, soybean, safflower, sunflower, coconut, palm, pumpkin, squash, sesame, poppy, grape, mung beans, peanut, peas, beans, radish, alfalfa, cocoa, coffee, hemp, walnuts, almonds, pecans, chick-peas potatoes, carrots, sweet potatoes, tomato, pepper, cassava, willows, oaks, elms, maples, apples, bananas, lilies, orchids, sedges, roses, buttercups, petunias, phlox, violets, and sunflowers.
23. A transformed whole plant or part thereof according to claim 20 wherein the plant cells, seeds or tissues are those of rice.
24. A method of transforming plant cells, seeds or tissues using isolated DNA molecules comprising nucleotide sequences comprising one or more expression cassettes capable of directing expression of two or three enzymes characteristic for the carotenoid biosynthesis pathway in plant cells selected from the group consisting of:
phytoene synthase derived from a bacteria and phytoene desaturase derived from fungi;

phytoene synthase derived from a bacteria and phytoene desaturase derived from bacteria; and,

phytoene synthase derived from a bacteria, phytoene desaturase derived from a plant and ζ -carotene desaturase derived from a plant.

25. A method according to claim 24 wherein the plant cells, seeds or tissues selected for transformation are normally carotenoid free.

26. A method according to claim 25 wherein the plant cells, seeds or tissues selected for transformation have a carotenoid content 0.001% w/w or lower.

27. A transformed whole plant or part thereof regenerated from transformants yielded according to claim 24.

28. A transformed whole plant or part thereof according to claim 27 selected from the group consisting of eukaryotic alga, embryophytes comprising *Bryophyta*, *Pteridophyta*, *Gymnospermae*, *Magnoliopsida*, *Rosopsida*, and *Liliopsida* ("monocots").

29. A transformed whole plant or part thereof according to claim 28 selected from the group consisting of rice, wheat, barley, oats, amaranth, flax, triticale, rye, corn, *Brassica* seeds, cotton seeds, soybean, safflower, sunflower, coconut, palm, pumpkin, squash, sesame, poppy, grape, mung beans, peanut, peas, beans, radish, alfalfa, cocoa, coffee, hemp, walnuts, almonds, pecans, chick-peas potatoes, carrots, sweet potatoes, tomato, pepper, cassava, willows, oaks, elms, maples, apples, bananas, lilies, orchids, sedges, roses, buttercups, petunias, phlox, violets, and sunflowers.

30. A transformed whole plant or part thereof according to claim 27 wherein the plant cells, seeds or tissues are those of rice.